On the Development of the Cape Cephalodiscus (C. gilchristi, Ridewood).

By

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With Plates 13 and 14.

In October, 1915, I recorded some observations on living specimens of the Cape Cephalodiscus, its eggs and larvæ (**4**). I had hoped in the following summer to be able to procure additional specimens, more especially of advanced larvæ, showing the process of metamorphosis. Contrary however, to all expectations, not a single living specimen was procured by the trawlers during the summer months. One colony, very much damaged, and with the zooid cavities filled with sand grains, was found on a sandy bottom, some six or eight miles from the usual habitat of the animal, of value only as indicating that the animal may be carried some distance by currents. The reason for this scarcity probably was, as suggested by the captains of the trawlers, that there had been no heavy seas, and no great "drawback" or strong currents to detach the colonies from the rocky ground which, there is reason to believe, is their natural habitat.

The material, procured during the previous summer, however, has proved on examination sufficient to indicate some new facts regarding the development of the animal, which it may be desirable to put on record, without waiting an indefinite time for the uncertain possibility of procuring and rearing the larvæ to later stages.

This material was preserved in a variety of ways. Sublimate, sublimate-acetic, Gilson's fluid, formalin-alcohol, alcohol, Fleming's fluid, and formalin in sea-water were employed. Sublimate preparations seemed to be unfavourably affected, more especially in the yolk-laden parts; osmic acid caused great contraction, though the fixing was good, at least in the larva; the best results were obtained from 10 per cent. formalin in sea-water, provided care was taken to pass the tissue slowly through gradations of absolute alcohol and xylol. The passage from absolute alcohol to xylol was best effected by using half a dozen gradations of these up to pure xylol, though good results were obtained by passing the material from absolute, through to one-third and two-thirds xylol to pure xylol. By the use of this method some very distinct preparations were procured, showing cellular structure, of the early stages within the egg capsule, though, for some reason, the method was not successful in larvæ hatched out from the egg.

The history of the investigation into the development of Cephalodiscus need not be here gone into, further than to indicate certain points on which further information is desirable, or which are not beyond dispute. Masterman (1900) observed some segmenting eggs in the material procured by the "Challenger" Expedition. Andersson (1903) noted the planula-like larva for the first time. Harmer (1905) described the eggs, heavily laden with yolk, their holoblastic and nearly equal segmentation, and suggested that they may give rise to solid embryos, in which the endoderm arises by delamination. He showed that the five body cavities of the adult arise at an early stage in the embryo. Andersson (1907) described his material more fully, and recorded the occurrence of a gastrula-like stage, in which there is a centrally placed mass of yolk with a narrow lumen; he believes that this is the endoderm formed by a process of invagination. He also confirmed the early appearance of the body cavities. Schepotieff (1909), though adding nothing further towards the elucidation of the gastrula stage, confirms the existence of the planula-like larva, and adds a further stage, which is free-swimming, and in which the five body cavities may be recognised. He regards the central mass of yolk in the embryo and larva as representing an endoderm formed by involution. Braem (1911) draws a comparison between this free-swimming larva and that of certain Polyzoa. Harmer (1915) draws attention to the different disposition of the body cavities, and gives a summary of the points in the development of Cephalodiscus on which there appears to be unanimity, drawing attention to the unexplained origin of the gastrula-like stage.

There are, as will be seen from this review, several important gaps in the development of Cephalodiscus which have not yet been filled up, and on which further information is very desirable. Thus, nothing is known of what occurs between the first segmentation stages and the gastrula—this alleged gastrula requires further investigation; the exact mode of origin of the body cavities has not been explained; and finally, the metamorphosis of the larva into the adult still remains to be elucidated.

The chief points on which the present work seems to throw further light with regard to these questions are: Segmentation stages leading to the formation of a blastula, which does not become invaginated to form a gastrula, but develops into a solid body, the outer parts of which become differentiated into an ectoderm and endoderm, the main inner yolk mass not representing the endoderm, nor its cavity the archenteron; the origin of the body cavities, the anterior as a part partitioned off from the archenteron, and the posterior as a lumen internal to the developing endoderm.

Ovary and Ovarian Egg.

The structure of the ovary of this species has been briefly described by Ridewood (7). It consists of a very narrow oviduct, leading into a mass of developing eggs in which no distinct lumen can be made out. The great majority of the eggs are comparatively small, one or two, at the point furthest from the oviduct, being greatly developed, and constituting the main part of the whole ovary. Each of these eggs is lodged in a follicle of small cells. Schepotieff (**8**, p. 81, fig. 63) has described the large ova of certain species as lying between a central epithelial lumen and the wall of the oviduct, where they are surrounded by a relatively large quantity of blood. This has not been observed in the present species, in which the ovum in its follicle is always in close contact with the surrounding ova. The ova appear to arise in the walls of the oviduct.

Two questions have been raised with regard to the ovary—the function of the pigment of the oviduct, and the method of discharge of the very large ova. With regard to the first, there is nothing new to add, except that the pigmented oviduct does not seem to be a luminous organ as has been suggested. None of the living animals examined, with a special view to ascertaining this, showed any trace of luminosity. With regard to the second question, Masterman's suggestion, with which Andersson (**2**, p. 86) does not agree, that the ova are set free on the death of the animal, seems to have some partial confirmation, from the fact that, in the fresh material, detached ovaries were frequently found. These may, of course, have been forcibly detached in the trawl, but living zooids were also observed in which the ovary, loosely attached to the animal, was seen to be quite exposed, suggesting that the whole ovary, or part of it, may break away from the body, without, however, necessarily involving the death of the animal—a condition which may also, perhaps, have been brought about by pressure in the trawl-net.

Certain histological features of the ovarian egg, which do not seem to have been noted, may be worthy of mention, as they seem to indicate that the subject is worthy of further study. The nucleus (Pl. 13, fig. 1) is a prominent feature of the developing egg. It is of a clear, almost homogeneous appearance, with only indistinct indications of chromatin elements. It has a distinctly demarcated border, which may, however, assume various irregular shapes, as if it were of an amœboid nature, though there were no prolongations into the surrounding yolk mass. It is never of the elongate or semilunar form figured by Schepotieff for his species. A conspicuous, deeply staining nucleolus is always present, and, in some preparations, was observed to have a series of rounded vacuole-like spots, arranged around its border; in other cases there appeared to be a single large vacuole, seemingly confirming the view that the vacuoles are of a changing nature. That the nucleolus takes a part in the functional activity of the egg at this stage is indicated by these different appearances, and also by the fact that in some cases it was observed drawn out in a tapering manner quite to the periphery of the germinal vesicle, and, in one case, a small detached part of it was observed lying in the germinal vesicle not far from it.

Stages in the formation of the yolk granules are well illustrated. These granules are very numerous, of an oval or rounded shape, with well-defined borders, and stain deeply with eosin. Scattered throughout them appeared a number of minute bodies (Pl. 13, fig. 1, *y. n.*), which readily stained with hæmatoxylin. The transformation of the homogeneous substance of the ovum into yolk granules does not appear to begin in the immediate neighbourhood of the nucleus, as in some other cases, for, in several instances, the nucleus with its nucleolus was observed in a homogeneous matrix in the form of a crescent at the periphery of a large mass of yolk granules. In others, the homogeneous part assumed the form of a portion slightly constricted off from the main mass of the egg. The boundary between the homogeneous and the granular part of the egg in these cases was well defined, and in it occurred a layer of the deeply staining bodies above mentioned, which may be termed yolk nuclei, though a variety of objects seem to be included under this term. So definite was the demarcation that it was at first supposed that there were here two cells, the semilunar homogeneous cell with its nucleus being a nourishing cell, assisting in building up the relatively enormous yolk mass. The fact that both were enveloped in a common follicle was not sufficient in itself to disprove this, but, as no nucleus could be found in the larger mass, it must be concluded that we are dealing here with one ovum. More advanced ova, completely transformed into yolk granules and yolk nuclei, possessed a large nucleus and nucleolus, located now in the centre.

The origin and nature of the yolk nuclei in general is still an obscure question. It has been suggested that they arise independently in the cytoplasm, that they are derived from the nucleus, and that they are derived from the nucleolus. The evidence in this case seems to be in favour of the last suggestion, in view of the appearances in the nucleolus noted above. The nuclei did not appear to originate from a single large yolk nucleus as is the case in other instances of such structures. The further study of the change in the ovarian egg seems to be worthy of attention.

The Fertilised Ovum.

Two or three specimens only of the unsegmented ovum, enclosed in its clear capsule and presumably fertilised, were procured. Such eggs (Pl. 13, fig. 2) were quite spherical, in contrast to later stages. In sections, among the numerous eosin-stained yolk granules, were seen some small bodies, stained, though not conspicuously, with hæmatoxylin, presumably the yolk nuclei.

The eggs of Cephalodiscus have been described as oval and of a varying diameter. These are probably late stages of the ovum, in which the embryo is fairly advanced, and the egg proper may not vary much in diameter, though sufficient material at this stage was not available to give any certainty on this point.

Segmentation.

The first division of the ovum, from the beginning of the constriction to the complete separation of the blastomeres, was observed. More examples of this stage were found than of the undivided egg, but still comparatively few (about ten out of several hundreds examined). This probably indicates that this stage is passed through at a comparatively rapid rate. Segmentation was in all cases total and usually about equal. A typical case is shown in Pl. 13, fig. 3, in which the blastomeres are about equal. Cases of decidedly unequal division, however, occurred as shown in Pl. 13, fig. 4, and in one case the smaller blastomere was ·21 × ·16 mm., the larger ·29 × ·37 mm. A large nucleus with nucleolus was conspicuous in some cases in each segment.

Stages of four blastomeres were about as numerous as those of two. In some the second division was of the typical form, equal and at right angles to the first (Pl. 13, fig. 5). In others there were decided departures from this type. Thus a stage was found (Pl. 13, fig. 6), in which the blastomeres did not lie in one plane, each of them being so placed that it was in contact with the other three, as if a relative change in position had taken place subsequently to the second division, or the division spindles of the second division had been at right angles to each other. A second aberrant type (Pl. 13, fig. 7) was found in two cases, in which two segments were widely separated, the other two being in close contact with each other. Both of these types may be connected with the fact, shown in another case, in which the division in one segment has been more rapid than in the other, resulting in the formation of three blastomeres, one large and two small (Pl. 13, fig. 8).

This segmentation may therefore be unequal, not only in quantity, but in point of time and method of division, probably connected with the great amount of yolk in the egg, a fact which also, as will be seen later, has a striking effect in further development. It apparently indicates a very indeterminate type of segmentation which is seen also in the next stage observed. This consisted of six cells (Pl. 13, fig. 9).

Blastula.

The earliest appearance of the segmentation cavity was at a stage showing six segments in section (Pl. 13, fig. 10); here it was very small, and was occupied by a homogeneous substance stained faintly with hæmatoxylin. A well-marked blastula is soon developed after this stage, for a section showing nine cells has a relatively large blastocœle (Pl. 13, fig. 11). Here the cells at one side appear larger than at the other. Other sections, however, show that the grouping of large cells at one point does not appear to be constant.

In a blastula of fourteen cells in section (Pl. 13, fig. 12) one cell was observed entirely within the hitherto complete circle of cells. It seems from subsequent events that this arises by proliferation of an outer cell rather than by ingrowth of a cell. It marks, as will be seen in later stages, the posterior end of the developing embryo.

The beginning of a further change is seen in a blastula of about twenty-nine cells in section (Pl. 13, fig. 13), in which a more marked polar disposition of parts becomes evident. At one end, which, as subsequent development shows, becomes the anterior end of the animal, the cells are decidedly elongate, while more posteriorly they are still rounded. The nucleus in the elongate cells appears at the distal end, while, in the rounded posterior cells, it appears in the centre.

Formation of the Yolk Columns.

The elongate outer cells begin to assume a columnar form, whose main body consists of an elongate mass of yolk cells with a peripherally placed nucleus (Pl. 13, figs. 14 and 15, *ex. y. c.*). This is probably due to their increase in number, and consequent mutual pressure. The elongate character is gradually assumed by the other cells in a more posterior position, and ultimately all the outer cells assume the form of columns with peripheral nuclei. The last of these outer cells to assume the columnar form is a group of rounded cells at the extreme posterior end, from which the internal cells are proliferating. The internal cells are still somewhat rounded, and ultimately completely fill the blastocœle, so as to form a solid mass of cells.

An interesting result of the rapid increase of these yolk columns is that an invagination is formed near the point (Pl. 13, fig. 15, *p. inv.*) where they are attached to the inner mass, apparently due merely to mechanical causes associated with the increase of the outer layer. This gives the appearance of a gastrula-like structure, which, however, can only be fully discussed when the changes in the inner yolk mass are considered.

Origin of Ectoderm.

After the formation of the external yolk columns, their cells divide rapidly, and the dermarcation between them, so clearly marked before, disappears. In view of their origin, we must regard each of the yolk columns as representing a single cell, and the breaking down of the cellular structure as due probably to the great abundance of yolk, the cell having lost control of the elongate and attenuated column. What was observed to occur at this stage was that the multiplying nuclei, with a certain amount of protoplasm, became each closely applied to a yolk granule, the two forming an ovoid body, in which the yolk granule, deeply stained with eosin, could be clearly distinguished from the nucleus, which was as distinctly stained with hæmatoxylin. Presumably the yolk granules serve as nourishment for the rapidly multiplying cells, for, ultimately, the yolk granules disappear, first from the peripheral parts, and later from the deeper parts of the ectoderm, till finally only a network of protoplasm, or rather a vacuolated protoplasmic mass, with numerous nuclei embedded in its substance, is left.

Two other points may be noted in connection with the origin of the ectoderm, viz. the formation of a basement membrane and the occurrence of excretory matter. With regard to the first, the outer cells were always distinguishable from the inner, except posteriorly, and an intervening space is seen in preparations of the more advanced stages. This demarcation becomes more distinct by the appearance of a fine basement membrane at the base of the ectoderm cells, apparently secreted by these cells (Pl. 13, fig. 17, *b. m.*). This basement membrane was not, however, usually so distinct as in the case figured, and it may be formed by the endoderm cells described later.

With reference to the second fact, there are to be seen in the developing ectoderm, after the cellular structure has been lost, a number of small bodies about the size of nuclei, but readily distinguished from them by their black colour. These become larger, and are frequently fused together to form elongate black masses. That they are ultimately passed out to the exterior was evident from some which were observed partly protruding beyond the surface of the developing embryo. This accounts for the presence in the living state of dark particles floating in the space between the embryo and the egg capsule, the rotation of the ciliated embryo causing them to move about rapidly, so that their presence is readily detected. It also accounts for the characteristic pigment spots of the embryo, which sometimes assumed an elongate shape, and formed a ring round the anteriorly situated sense organ.

Certain areas of the ectoderm seem to retain their distinctly cellular structure throughout the changes which take place in the ectoderm. The most prominent of these is the part which appears as a ventral thickening in the embryo (Pl. 13, fig. 18, *v. th.*), and which may, as Harmer suggests, represent the disc-like face of the proboscis. Its early appearance is noteworthy. The sense organ also consists of independent cells, as also the glandular cells of the ectoderm, but these are not seen at this early stage, and their cellular condition may be of much later origin.

Origin of Endoderm.

The origin and mode of formation of the endoderm is, as already indicated, one of the outstanding problems of the development of Cephalodiscus.

The cells of the ectoderm before fusion are arranged radially, and are at this stage clearly marked off from the inner cells; a little later they are further marked off from them by the basement membrane. Soon after the fusion of these ectodermal cells a few cells appear below the basement membrane. These are closely applied to yolk granules, and form with them a distinct layer round the anterior end of the yolk mass, but clearly marked off from it (Pl. 13, figs. 17, 18 and 19, *end.*). As the yolk granules in this layer are used up, each of the cells sends out a long protoplasmic process towards the other, so that, ultimately, they form a chain of attenuated cells devoid of yolk granules. These cells appear first at the anterior end and later more posteriorly, so that the chain of cells gradually extends backwards, over the internal yolk mass, as an uninterrupted series, to the posterior end, at the point where the internal cells remain in connection with the ectoderm.

Formation of the Yolk Cavity and Andersson's "Gastrula."

Meanwhile a change has taken place in the inner cells, associated perhaps with the appearance of the endoderm. Unlike the ectodermal cells, they do not assume a columnar form, but remain more or less rounded, each, however, with a nucleus and a distinct cell boundary, and as heavily laden with yolk granules as the ectodermal cells. On the appearance of the endodermal cells their cellular structure can no longer be distinguished. It appears as if the nuclei, with their associated protoplasm, no longer controlled these cells, and had wandered to the periphery, as in the case of the ectodermal cells, leaving a central non-cellular mass of yolk granules.

All the cells of the inner mass do not pass to the periphery, but some find their way to the centre, where they form a small but distinct group, embedded in a substance nearly devoid of yolk granules. A small cavity then appears in this substance. The cavity as seen in sections is usually rounded or oval in shape, but that it is in reality of an elongate nature is evident from the fact that it can be followed through a series of consecutive sections. It ends abruptly when traced in one direction, but may be followed in the other direction to the periphery of the embryo, where it suddenly ends in a shallow pit in the ectoderm, apparently the involution or invagination of the ectoderm already noted. This was most clearly seen in sections which passed through the axis of this part of the embryo (Pl. 13, fig. 16). The whole assumed the form of a structure, which, without this explanation of its origin, might be taken to be a typical gastrula, in which the central yolk-laden mass represents an endoderm, formed by invagination, and a central cavity, the archenteron; the only suspicious feature being the very narrow lumen and the absence of cellular structure of the endoderm.

Andersson (**2**, p. 87) was the first to notice and figure this gastrula, and he has apparently no doubt as to how it has arisen. He notes Harmer's suggestion (**5**, pp. 109, 110) that the gastrula is probably formed by a process of delamination, and considers that for his species at least this is not the case, but that "die Gastrula durch eine typische Invagination sich bildet" (p. 89). He was unfortunately unable, he adds, to carry out any study of the cellular structure, as owing, he believes, to imperfect preservation, the ectoderm and endoderm appeared uniformly filled with yolk granules, which he notes, however, were absent in the immediate vicinity of the central lumen. His figure, however, indicates the existence of the external yolk columns. Schepotieff (**8**, p. 437) states that he found gastrula stages in C. indicus, but was unable to follow out their formation. That, however, he accepts the view that the central yolk mass represents an endoderm formed by invagination, is apparent from his description and figures of larval stages of the species. Harmer also (**4**, p. 245) accepts the view that the central yolk represents the wall of the archenteron.

It appears from what has been observed that, in this species, the central yolk-laden cells arise solely by unipolar proliferation of cells into the cavity of the blastula, that the cellular structure breaks down, and some of the nuclei, with their associated protoplasm, go to form the endoderm, while others pass towards the centre and become vitellophags. Owing to the activity of these latter the yolk granules become used up, leaving a homogeneous detritus in which a cavity subsequently appears. This cavity extends at first to an ectodermal involution, and it may be that the excretory matter passes out in this way to the exterior, just as the excretory products of the growing ectoderm are given off in another manner already indicated. The subsequent changes in this cavity and its relation to the posterior involution, as well as to the cavity immediately enclosed by the endoderm, will be described in later stages.

Formation of Internal Yolk Columns.

After the inner yolk-laden cells become a homogeneous mass of yolk with scattered cells, and soon after these reduced cells begin to migrate, some towards the periphery, some to the centre, a change takes place in the uniform distribution of the yolk granules, and they assume the form of a number of yolk columns, or rather pyramids, whose apices meet round the central cavity, and whose broader distal extremities are in the proximity of the cells which form the endoderm (Pl. 13, figs. 18 and 19, *i. y. c.*). The result bears some resemblance to what has occurred in the ectoderm, but it has apparently been attained in a different way, for the large yolk-laden cells do not individually become yolk columns; at least there was no appearance of this, and it can hardly be imagined how they could do so, unless perhaps the cells, migrating outwards to form the endoderm, retained for a time some control over their original yolk masses, and similarly the cells migrating inwards draw out their associated yolk into lenticular masses.

The functional significance of the whole process seems very evident. A very little of the yolk is necessarily used up in the formation of the thin endodermal layer, and the main mass is reserved to be transformed by vitellophags into a form which can be absorbed by the archenteron to feed the rapidly developing ectoderm, which has now used up its original supply of yolk.

The inner yolk pyramids persist as such for a considerable time, but later, when their yolk granules have been much reduced, they seem to disappear.

Origin of Body Cavities.

As development proceeds and the anterior part of the endoderm increases in size, a space (the archenteron) appears between it and the central yolk mass (Pl. 13, fig. 19, *arch.*). Posteriorly the endoderm is still in close contact with the yolk mass, but later a few cells, evidently arising from the yolk mass, as the endodermal cells did, appear below it on the yolk. These increase in numbers and ultimately form a distinct layer, so that the endoderm here seems double. The cavity between these two layers is very evidently the beginning of the first pair of posterior body cavities (Pl. 13, fig. 19, and Pl. 14, fig. 20, $b.\ c._2$). The second pair of body cavities is subsequently formed in a similar manner (Pl. 13, fig. 18, and Pl. 14, fig. 20, $b.\ c._3$). The body cavities may therefore be regarded as of endodermal origin, which, though not typically enterocœlic, is a modified form of such a method of development. At later stages both pairs of body cavities may be seen with a complete epithelial lining (Pl. 14, fig. 21, $b.\ c._2$ and $b.\ c._3$).

The definite origin of the single anterior body cavity was not observed until later, but it may be mentioned here that it is developed from the anterior part of the archenteron.

The Larva.

The structure of the larva soon after hatching is not very different from that of the late embryo, but certain points, obscured by the compression of the embryo in a small space, become clearer or assume a different aspect in the early larva. Thus the ectoderm, very much folded in the embryo, now expands, and the body cavities can more readily be made out. Certain more definite changes, however, were observed in older larvæ.

The general structure of the larva has already been described by Harmer (**5**), Andersson (**2**), and Schepotieff (**8**). The chief new points to be added are in connection with (1) the fate of the internal yolk mass, (2) the arrangement of the body cavities and the mode of origin of the anterior body cavity, (3) the origin of the anus, (4) the involution of the sense organ and its nervous tissue, and (5) a postero-ventral thickening and involution.

(1) Fate of yolk mass.—Neither Harmer nor Andersson found any trace of cellular structure in the yolk. Schepotieff, however, indicates clearly (**8**, Pl. 8 fig. 16) that this part is divided up into large columnar cells, which, but for their distinct demarcation and single nuclei, might pass for the internal yolk columns, already described for the species under consideration. His fig. 7 also shows the walls of what he regards as the proboscis-cœlom, ending abruptly at the anterior part of the yolk, instead of passing round it to the posterior extremity, as here described. The cells of this body cavity are obviously very diagrammatically drawn, and it may be that those of the "Urdarm" in fig. 16 are of the same nature, in which case it would not be so difficult to interpret them as internal yolk columns. It is not, however, absolutely necessary to reconcile other accounts of the formation of the endoderm with that given here, as both may be correct; the mode of development, even in closely related forms of animals, having been proved in some cases to be very different.

The further changes observed in the central yolk mass were as follows: The anterior space (archenteron) between the endoderm and the yolk becomes very large, and at the same time the yolk lumen increases in size (Pl. 13, fig. 19, *y. l.*).

This lumen appears somewhat triangular in longitudinal section; in sagittal sections of some advanced embryos it is seen that the part of the yolk forming the upper portion or roof of the central cavity has disappeared, and it is now in connection dorsally with the archenteron. The consumption of these yolk granules in this particular region is apparently associated with the active growth in the tissues immediately adjacent to it. More posteriorly, a part of the roof of the lumen is still present, and a transverse section of this part would show a circular cavity in the yolk. This is very well illustrated in Andersson's figs. 73–78, and in transverse sections of younger larvæ of this species. He still regards this diminishing cavity as the archenteron, and its walls, including the homogeneous substance which is interpreted here as the detritus of yolk granules attacked by vitellophags, as the endoderm formed by invagination. The very large cavity in front of and above the yolk can in this case only be considered, as he does, to represent the cavity of the proboscis. Later embryos, however, show that the roof of the yolk lumen disappears even from the posterior part, just as it did in the anterior, so that no yolk cavity is left at all. This is seen in transverse sections (Pl. 14, figs. 23–30) of an advanced larva the exact age of which cannot be determined; it was found crawling over a cœnœcium and kept alive for about two days afterwards. Here the roof of the yolk lumen has entirely disappeared, though the floor, or ventral part, still persists as a fairly large mass, with the cavity of the archenteron above it. The ventral part of the yolk is probably used up in the next step in the metamorphosis of the larva, as yet unknown, but probably most marked on the postero-ventral aspect of the larva, where this yolk mass lies.

(2) The body cavities of the larva.—The four posterior body cavities can usually be seen distinctly in suitably prepared material, provided there is not too great contraction of the tissues. Their epithelial lining can also be sufficiently distinguished. As they are of importance in the

organisation of Cephalodiscus, and as their relative position and extent may have a bearing on the subsequent processes of metamorphosis, some details may be added to what is already known for other species.

Only a limited number of larvæ were available, and several of these were, for various reasons, unsuitable for detailed examination of the cavities, some being too contracted or distorted, others were somewhat broken up, owing to the difficulty of getting whole sections through the yolk mass. One or two series of transverse sections were, however, satisfactory, and showed the body cavities clearly. In one of these, cut into a series of 140 sections, the sixth from the posterior end (Pl. 14, fig. 22) showed that the two posterior body cavities extended backwards beyond the yolk and archenteron. At the 17th section (Pl. 14, fig. 23) the body cavities are very large, extending almost completely round the yolk, but are separated from each other dorsally by a mesentery and ventrally by the posterior thickening already mentioned. That there is a here a ventral mesentery obscured by the pressure of the yolk is shown in other series, and it is evident in the next or 24th section (Pl. 14, fig. 24). Here the upper wall of the archenteron has become broadly attached to the ectoderm, and the body cavities are beginning to disappear from the dorsal side. A few sections further on, at the 25th, the beginning of the second body cavity appears on the right side at its dorso-lateral corner, and, at the 27th section (Pl. 14, fig. 25), it is of considerable size. At the 29th section (Pl. 14, fig. 26) it has extended to the right side of the archenteron, and in this section the second body cavity of the left side appears at the upper angle formed by the ectoderm and the wall of the archenteron. That the point at which the second body cavities begin on each side is therefore not similar is evident from this, and in other series of sections it also shows a variation, as, for instance, in one in which it begins quite at the lateral wall of the ectoderm. The 36th section (Pl. 14, fig. 27) shows further advance, and, at the 45th (Pl. 14, fig. 29), the third body cavity has disappeared on the right side. In the 67th section (Pl. 14, fig. 30) both pairs of body cavities have disappeared. At the 95th section (Pl. 14, fig. 31) the yolk mass is much smaller, and the ectoderm has the clear spaces and the elongate cells characteristic of the dorsal and ventral parts of this region of the body respectively.

The formation of the definite body cavity of the proboscis was not seen in these transverse sections, but in some longitudinal sections a division appeared running obliquely across the cavity of the archenteron anteriorly, and cutting off a portion of this cavity, the portion cut off being about one-fourth of the whole archenteron. This division was observed in two longitudinal sections only, and in these the thin wall of the archenteron was incomplete in places (Pl. 14, fig. 37). How this division arose is not quite clear, and the question is perhaps better left open till further confirmation is possible.

(3) Appearance of the anus in the larva.—In a sagittal section (Pl. 14, fig. 38) of a larva the cavity of the archenteron extends to the posterior end, and comes in contact with the ectoderm. At the point of contact there is a slight involution and indication of a pore, though there is no well-marked opening. There seems little reason to doubt that this is the point of origin of the anus. In the section it is situated towards the dorsal aspect of the body. It doubtless, therefore, represents the point at which the yolk mass remains in contact with the ectoderm, but has no connection with what is described later as a postero-ventral thickening and involution of the ectoderm. The section, however, was not entirely convincing, and later stages are desirable to clear up and confirm this point. There is no indication of the anal opening in Pl. 14, fig. 22, a transverse section posterior to yolk and archenteron.

(4) Changes in the sense organ appear in the larva. In the earlier stages it consisted of a group of elongate ciliated cells, at the base of which appeared a small patch of nervous tissue, as described for other species. In the more

advanced larvæ the nervous tissue is seen to extend in a posterior direction under the general tissue of the ectoderm (Pl. 14, fig. 32), and the cells of the sense organ now assume the same character as this nervous tissue. They lose their cilia, and become sunk in an ectodermal pit (Pl. 14, fig. 33), which may be drawn out posteriorly into a tubular structure (Pl. 14, fig. 35, *inv. s. o.*).

(5) A postero-ventral thickening and involution of the ectoderm was observed in some sections under the hinder end of the yolk mass. This thickening is seen in Pl. 14, fig. 23, and, a few sections posterior to it, it is seen to lead to an involution. This involution is, however, more clearly seen in another series (Pl. 14, fig. 36). It may not prove to be of any particular significance, but may be noted, as it is in this region that the greatest change will probably take place in the metamorphosis of the larva.

Summary of Results.

(1) Certain facts are noted with regard to the formation of yolk granules, presence of yolk nuclei, character of nucleus and nucleolus.

(2) The segmentation is holoblastic, equal, or markedly unequal, and apparently indeterminate.

(3) A blastula stage occurs.

(4) The blastula becomes solid by proliferation of cells at one end; there is no invagination at this stage.

(5) The point of proliferation marks the posterior end, and the anterior end is distinguished by the elongation of its cells.

(6) All the outer cells become elongate, and assume the character of columnar cells full of yolk. As these increase in number a small posterior invagination appears.

(7) The cellular character of the yolk columns disappears; the yolk granules are used up, and an ectoderm consisting of many nuclei in a protoplasmic network, with a basement membrane, is formed.

(8) Excretory matter in the form of dark specks and elongate rods is formed during this process and constitutes the characteristic pigment of the late embryos and larvæ.

(9) The ventral thickening of the ectoderm is found at an early stage.

(10) The endoderm appears under the ectoderm, first as a number of cells at the anterior end, and ultimately as a complete chain of cells extending over the inner yolk, except at the point of proliferation at the posterior end.

(11) The cells occupying the blastocœle break down like the outer cells, and become a mass of yolk granules, in which are scattered a number of nuclei with associated protoplasm.

(12) Some of these pass outward to form the endoderm, others pass inwards to form vitellophags.

(13) A lumen is formed in the yolk mass and it becomes connected to the posterior involution, giving rise to a gastrula-like structure.

(14) The internal mass of yolk assumes the form of a number of yolk columns or pyramids.

(15) The posterior body cavities arise by a number of cells from the yolk mass forming a second layer under the endodermal layer.

(16) The yolk lumen increases in size, the yolk granules becoming converted into a homogeneous substance. This takes place chiefly on the dorsal side, where the yolk lumen becomes connected with the archenteron.

(17) The position and extent of the five body cavities in the larva are shown.

(18) The yolk in the larva is in the form of an elongate mass of granules and homogeneous matter, lying on the floor of the archenteron.

(19) Changes are described in the larval nervous system, and the appearance of a posterior thickening and involution of the ectoderm below the yolk mass is noted.

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EXPLANATION OF PLATES 13 AND 14,

Illustrating Dr. J. D. F. Gilchrist's paper "On the Development of the Cape Cephalodiscus (C. gilchristi, Ridewood)."

Explanation of Figures.

The following reference letters are used in the figures: *an.* Anus. *arch.* Archenteron. *b. m.* Basement membrane. *d.* Yolk detritus. *end.* Endoderm. *ex. y. c.* External yolk columns. *i. y. c.* Internal yolk columns. *inv. s. o.* Involution in nervous tissue of sense organ. *N.* Nucleus. *n.* Nucleolus. *p. inv.* Posterior invagination. *p. v. inv.* Postero-ventral involution. *p. v. th.* Postero-ventral thickening. *s. o.* Sense organ. *vit.* Vitellophag. *v. th.* Ventral thickening. *y.* Yolk. *y. gr.* Yolk granules. *y. l.* Yolk lumen. *y. n.* Yolk nucleus.

[All the figures have been drawn by camera lucida except figs. 5.

and 8; figs. 1, 17, and 20 with a Zeiss F objective; figs. 2–13 with a Zeiss A, and the remainder with a Zeiss C. The ectoderm is represented diagramatically by a light shading, the yolk granules by a stippled shading, where details are unnecessary. The scale of magnification is shown by a line representing 50 μ.]

PLATE 13.

Fig. 1.—Section of ovarian egg. *N.* Nucleus. *n.* Nucleolus. *y. gr* Yolk granules. *y. n.* Yolk nucleus.

Fig. 2.—Fertilised ovum.

Fig. 3.—Two-celled stage with nearly equal division.

Fig. 4.—Two-celled stage with unequal division.

Figs. 5–7.—Four-celled stage showing various methods of division.

Fig. 8.—Three-celled stage.

Fig. 9.—Six-celled stage.

Fig. 10.—Section of egg showing 6 blastomeres and segmentation cavity.

Fig. 11.—Section of blastula showing blastocœle and contents.

Fig. 12.—Section of blastula showing beginning of internal proliferation at posterior end.

Fig. 13.—Section of blastula showing elongation of cells at anterior end of embryo.

Fig. 14.—Section showing a solid embryo, the blastocœle being filled with cells from the posterior proliferation. The external cells assume the form of external yolk columns (*ex. y. c.*).

Fig. 15.—Longitudinal section of an embryo showing the external yolk columns in increased numbers, and an invagination at the posterior end.

Fig. 16.—Section of gastrula-like structure showing vitellophags (*vit.*), homogeneous detritus (*d.*), yolk lumen (*y. l.*), posterior invagination (*p. inv.*), and traces of external yolk columns (*ex. y. c.*), now disappearing at the anterior end.

Fig. 17.—Section of part of anterior end of embryo showing the formation of the endoderm (*end.*), and the appearance of a basement membrane (*b. m.*).

Fig. 18.—Longitudinal section of an embryo showing the formation of inner yolk columns (*i. y. c.*), the further development of the endoderm (*end.*), and the early appearance of the ventral thickening (*v. th.*).

Fig. 19.—Longitudinal section showing further development of endoderm, and formation of posterior body cavities ($b. c._2$ and $b. c._3$),

increase in yolk lumen (*y. l.*), and disappearance of inner yolk columns from dorsal region of yolk lumen.

PLATE 14.

Fig. 20.—Longitudinal section showing details of formation of posterior body cavities, a second layer of cells forming the inner wall of $b.\,c._2$ but not yet in $b.\,c._3$.

Fig. 21.—Horizontal section showing the completed epithelial lining of $b.\,c._2$ and $b.\,c._3$.

Figs. 22–34.—Transverse sections selected from a series of 140 of a larva to show the positions and relations of the posterior body cavities ($b.\,c._2$ and $b.\,c._3$), the archenteron (*arch.*), the yolk (*y.*), and the sense organ (*s. o.*).

Fig.	22	is the	6th	from the	posterior end.
,,	23	,,	17th	,,	,,
,,	24	,,	24th	,,	,,
,,	25	,,	27th	,,	,,
,,	26	,,	29th	,,	,,
,,	27	,,	36th	,,	,,
,,	28	,,	40th	,,	,,
,,	29	,,	45th	,,	,,
,,	30	,,	67th	,,	,,
,,	31	,,	95th	,,	,,
,,	32	,,	123rd	,,	,,
,,	33	,,	132nd	,,	,,
,,	34	,,	138th	,,	,,

Fig. 35.—Transverse section from another series showing involution (*inv. s. o.*) in nervous tissue of sense organ below ectoderm.

Fig. 36.—Transverse section showing postero-ventral thickening (*p. v. th.*), and involution (*p. v. inv.*).

Fig. 37.—Longitudinal vertical section of larva showing the anterior body cavity ($b.\,c._1$).

Fig. 38.—Longitudinal vertical section of posterior end of larva showing origin of anus (*an.*).